

## Influence of Nitrogen Availability on Aminotransferases in *Lemna minor* L.

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### ABSTRACT

Protein contents and glutamate:glyoxylate, serine:glyoxylate, alanine:glyoxylate and glutamate:pyruvate aminotransferase activities per gram fresh weight declined sharply when *Lemna minor* L., previously grown on nitrate medium, was starved of nitrogen. Nitrogen replenishment after 5 d caused complete recovery of these parameters with higher values in ammonium-fed than nitrate-fed plants 7 d after transfer of plants from nitrogen-free medium.

Glutamate:glyoxylate and alanine:glyoxylate aminotransferase specific activities (based on total extracted protein) showed little change with nitrogen availability. Serine:glyoxylate aminotransferase increased slowly during nitrogen starvation and decreased following nitrogen replenishment whether with ammonium or nitrate. After 1 d of nitrogen starvation the specific activity of glutamate:pyruvate aminotransferase declined; it increased following nitrogen replenishment and ammonium gave rise to a greater activity than nitrate.

The results are discussed in relation to the differences in stability of the various enzymes relative to the overall protein turnover rate.

**Key words:** Aminotransferases; Nitrogen source; Photorespiration.

### INTRODUCTION

The presence or absence of exogenous nitrogen profoundly influences the pattern of carbon assimilation in photosynthetic plants. Photosynthetic carbon reduction (PCR) and photorespiratory carbon oxidation (PCO) cycles and oxidative phosphorylation are affected (Blackwood and Mifflin, 1976; Habib Mohamed and Gnanam, 1977; Platt, Plaut, and Bassham, 1977; Marques, Oberholzer, and Erismann, 1983; Fuhrer and Erismann, 1984). The general effect of nitrate and ammonium is to increase incorporation of assimilated carbon into amino acids at the expense of starch and sucrose (Blackwood and Mifflin, 1976; Habib Mohamed and Gnanam, 1977; Platt *et al.*, 1977). There is evidence that the interaction of carbon and nitrogen assimilation is regulated by induction or repression of key enzymes according to the type and availability of nitrogen. In *Chlorella sorokiniana* the activities of enzymes involved in the initial stages of incorporation of ammonia into amino acids are altered by the introduction or removal of inorganic nitrogen or by change from ammonium to nitrate in the growth medium or the reverse (Tischner and Lorenzen, 1980).

The pathway of ammonium assimilation in *Lemna minor* L. depends on the availability of this substrate in the culture medium. Glutamine synthetase (GS) and glutamate synthase (GOGAT) activities were high in plants grown on low ammonium concentrations; increasing

the ammonium concentration decreased the activities of these enzymes and increased glutamate dehydrogenase (GDH) activity (Rhodes, Rendon, and Stewart, 1976). Glycolate oxidase, a major enzyme in the PCO cycle was doubled in specific activity and in molecular weight in ammonium-grown plants compared with those grown on nitrate (Emes and Erismann, 1982). Other enzymes directly or indirectly involved in PCO include the glutamate:glyoxylate (E.C. 2.6.1.4.), serine:glyoxylate (E.C. 2.6.1.45), glutamate:pyruvate (E.C. 2.6.1.21) and alanine:glyoxylate (E.C. 2.6.1.44) aminotransferases (GGAT, SGAT, GPAT and AGAT respectively; AGAT activity may not be due to a single specific enzyme). We report the effects of nitrogen starvation and its replenishment by ammonium or nitrate on these aminotransferase activities in *L. minor*.

## MATERIALS AND METHODS

### *Growth conditions*

*Lemna minor* L. was cultured for 7 d in Fernbach flasks under continuous ambient aeration on a modified Hutner nitrate medium (Hutner, 1953; Emes and Erismann, 1982) prior to transfer to nitrogen-free, ammonium or nitrate test media. The nitrogen concentration in the media containing this element was  $7.0 \text{ mmol N dm}^{-3}$ . Growth temperature was  $25^\circ\text{C}$  and continuous illumination at  $230 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  was provided by Philips TL 32 and Sylvania 'Gro-Lux' F 40 T 12-GRO fluorescent tubes. To ensure steady state conditions for growth, plants were thinned out and transferred to fresh nutrient media at intervals of 3–5 d. Nutrient solution pH values, which were monitored frequently, remained within the range 4.65–6.90.

### *Sampling of plant material*

In excess of 1.0 g plants was sampled from each flask. The adhering nutrient medium in each sample was washed away under vacuum filtration with distilled water and remaining surface moisture was removed by centrifugation.

### *Protein extraction*

One gram (fresh weight) of tissue was homogenized in  $4.0 \text{ cm}^3$  ice-cold  $10 \text{ mol m}^{-3}$  sodium borate buffer, pH 8.0, with the aid of a Polytron homogenizer, and filtered through one layer of Miracloth. The filtrate was centrifuged at  $24\,000 \times g$  for 15 min at  $4^\circ\text{C}$ . Desalting of the supernatant was conducted by the centrifugation of a  $1.5 \text{ cm}^3$  aliquot through a column of Sephadex G25 (Pharmacia), previously equilibrated with  $10 \text{ mol m}^{-3}$  borate buffer.

### *Aminotransferase assay procedure*

To  $100 \text{ mm}^3$   $1000 \text{ mol m}^{-3}$  sodium borate buffer, pH 8.0, in a test tube, was added  $100 \text{ mm}^3$  each of  $100 \text{ mol m}^{-3}$  sodium glyoxylate or pyruvate,  $200 \text{ mol m}^{-3}$  L-glutamic acid (dissolved and adjusted to pH 8.0 with sodium hydroxide), L-serine or L-alanine, double distilled water and plant protein extract. All assay components were mixed at  $0^\circ\text{C}$  and immediately following this aminotransferase reactions were started by immersion of the tubes in water at  $30^\circ\text{C}$ . Incubations were terminated 30 min later by returning the tubes to ice-cold water. Controls contained  $100 \text{ mm}^3$  water instead of extract. Proteins and cations were removed from  $200 \text{ mm}^3$  samples of reaction mixtures by passage of each of these through a  $1.7 \text{ cm} \times 0.5 \text{ cm}$  Dowex 50 (hydrogen form) column (bed volume  $0.33 \text{ cm}^3$ ) followed by four  $200 \text{ mm}^3$  portions of double distilled water. Keto acids in the filtrate were separated by high performance liquid chromatography (HPLC, Spectraphysics model 8000 B; Bio-Rad column for organic acid analysis,  $300 \text{ mm} \times 7.8 \text{ mm}$ , packed with Aminex HPX-87; eluant  $1.0 \text{ mol m}^{-3} \text{ H}_2\text{SO}_4$ ; flow rate  $0.6 \text{ cm}^3 \text{ min}^{-1}$ ; temperature  $15^\circ\text{C}$ ). The effluent was monitored spectrophotometrically at 210 nm with a Perkin Elmer LC 75 detector.

### *Protein assay procedure*

Samples of plant extracts were mixed with equal volumes of  $100 \text{ mol m}^{-3}$  sodium hydroxide and stored frozen; protein present was estimated by the method of Bradford (1976) using Bio-Rad colour reagent and bovine albumin (Fluka AG, fraction V) as a standard.

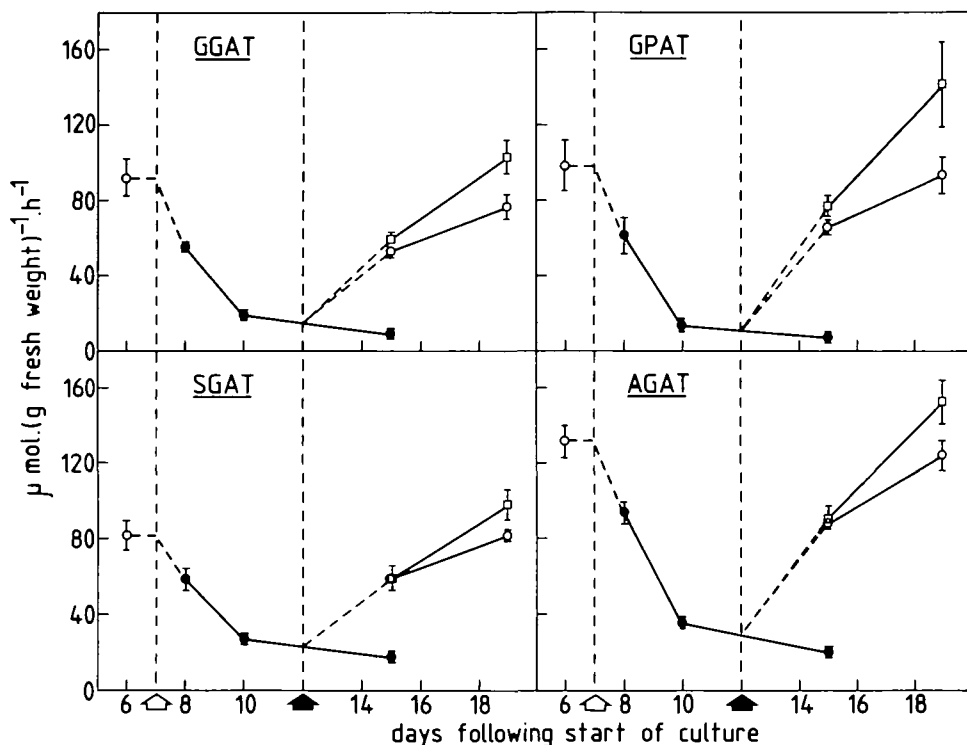


FIG. 1. Timecourse showing the effects of nitrogen starvation and its subsequent replenishment on fresh weight-based activities of *Lemna minor* L. aminotransferases. Plain and filled arrows respectively indicate the transfer of plants from  $\text{NO}_3^-$  (○) to  $\text{N}$ -free medium (●) and the transfer from the latter to  $\text{NH}_4^+$  (□) or  $\text{NO}_3^-$  (○). Each point plotted is the mean  $\pm$  s.d. for samples from nine separate culture flasks up to and including day 10 and three flasks from day 15 onwards.

## RESULTS

Plants were grown on nitrate medium in nine culture flasks and then transferred to nitrogen-free medium for 5 d. Flasks were divided into three groups of three for transfer either to nitrogen-free medium or to nitrate or ammonium media. Figure 1 shows timecourses of GGAT, SGAT, GPAT and AGAT activities in relation to nitrogen treatment on a tissue fresh weight basis. All four enzyme activities declined following transfer from nitrate to nitrogen-free media. This also produced frond yellowing and a deceleration of growth; insufficient biomass was available for sampling after 8 d of nitrogen starvation compared to treatments returned to nitrogen. These changes were reversed following the return of plants to nitrogen-containing media. Finally there were higher enzyme activities in the ammonium-grown plants than in the nitrate-grown plants.

Changes in aminotransferase activities were accompanied by changes in protein contents (Fig. 2). Three days following the return of plants to nitrogen, protein contents in plants given nitrate were similar to those in plants given ammonium; 7 d following nitrogen replenishment, protein contents were higher in plants given ammonium. This confirmed a preliminary experiment where *L. minor* plants, grown on nitrate, were transferred directly to ammonium or nitrogen-free media or retained on nitrate medium (data not shown).

Since protein contents changed with nitrogen availability aminotransferase activities are also plotted per unit protein (Fig. 3). Nitrogen starvation slightly increased SGAT specific

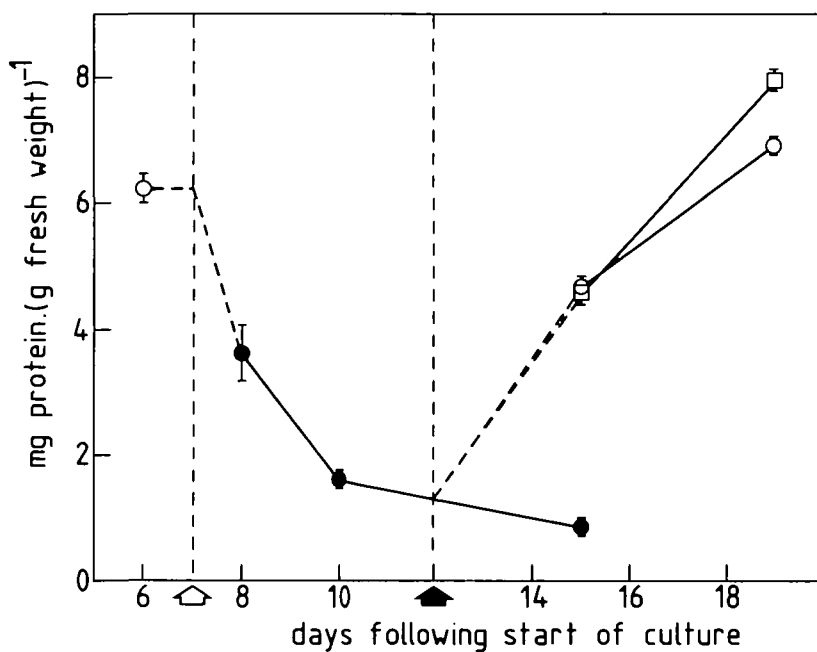


FIG. 2. Timecourse showing the effects of nitrogen starvation and its subsequent replenishment on soluble protein content of *L. minor*. Legend as for Fig. 1.

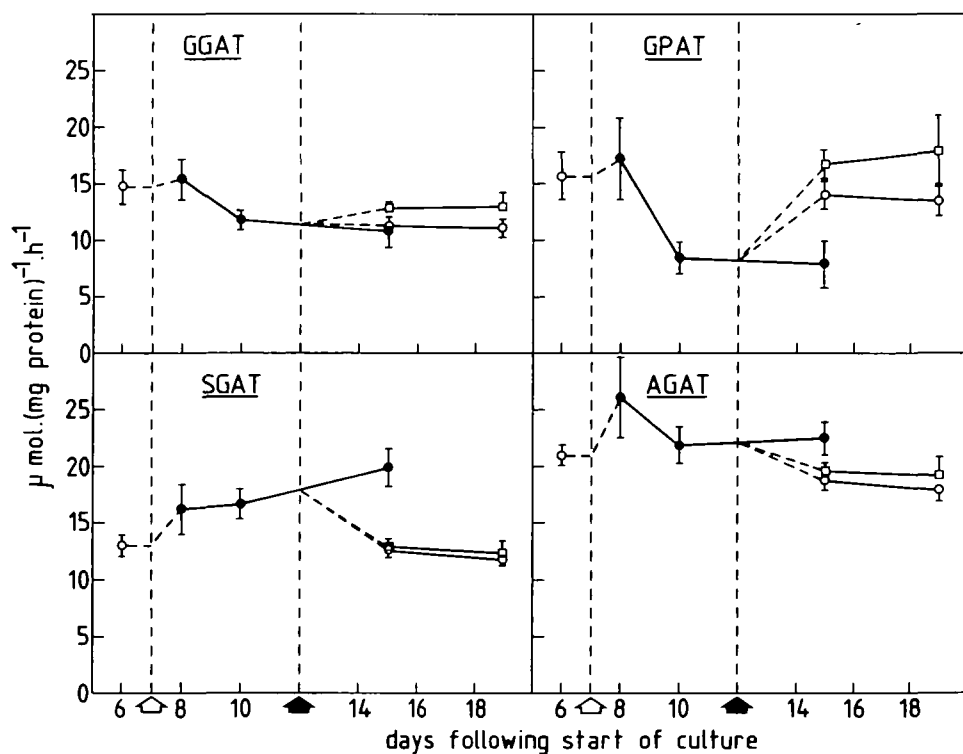


FIG. 3. Timecourse showing the effects of nitrogen starvation and its subsequent replenishment on specific activities of *L. minor* aminotransferases. Legend as for Fig. 1.

activity (based on total extractable proteins) over a period of 8 d; it decreased slightly again in plants returned to either the medium with nitrate or that with ammonium. After 1 d of nitrogen starvation GPAT activity declined sharply. The changes in specific activity of GPAT were the inverse of those of SGAT. However, 3 d following replenishment with nitrogen GPAT specific activity in ammonium-fed plants was greater than in nitrate-fed plants. GGAT specific activity showed similar, but considerably less well-marked trends to that of GPAT. After 5 d of nitrogen starvation AGAT responded like SGAT to the return of plants to nitrogen. Final values for all four aminotransferase activities in nitrate-fed plants (Figs 1 and 3), were similar to those prior to nitrogen starvation.

## DISCUSSION

The decline of protein contents under nitrogen starvation and their recovery following nitrogen replenishment in *L. minor* indicate active and possibly rapid protein turnover in this species. The fall in protein contents per gram fresh weight in the absence of nitrogen may be due to actual protein degradation or a dilution of protein by continued, although rapidly declining production of biomass. Davies and Humphrey (1978) observed an increase in protein turnover and amino acid recycling in nitrogen-starved *L. minor*. These authors suggested that this would facilitate a rapid alteration of the plant's enzyme complement in response to environmental change. Such a response to nitrogen starvation would be an increase in activities of enzymes maximizing the plant's nitrogen retaining abilities and conservation of enzymes maintaining carbon flux. Following transfer of *Chlorella sorokiniana* to nitrogen free medium, Tischner and Lorenzen (1980) observed an increase in GS, GOGAT and GDH activities, these enzymes being responsible for refixation of ammonia released in PCO (Keys, Bird, Cornelius, Lea, Wallsgrove, and Mifflin, 1978). In the present investigation none of the specific activities of those aminotransferases involved in PCO (SGAT, GGAT, AGAT) were decreased to any marked extent by nitrogen starvation; SGAT was slightly increased by this treatment (Fig. 3). Under the growth conditions used, photosynthesis must be accompanied by considerable glycolate and glyoxylate production, regardless of nitrogen availability, and therefore PCO aminotransferases will always be necessary for their metabolic removal. GPAT specific activity declined after 1 d of nitrogen starvation but increased following nitrogen replenishment (Fig. 3). This may relate to changes in chlorophyll contents with nitrogen availability. Hedley and Stoddart (1971) suggested an involvement of GPAT in the production of the chlorophyll precursor,  $\delta$ -aminolaevulinic acid, in *Lolium temulentum* leaves.

The changes in aminotransferase activities may be due to differences in enzyme turnover rates relative to overall protein turnover. The responses of SGAT and GPAT to nitrogen availability could be explained in terms of higher and lower *in vivo* stabilities relative to the average protein stability. This hypothesis assumes each of the aminotransferase activities studied here to be due to a single specific protein. However, studies with purified enzymes have shown that the glyoxylate aminotransferases do not all show absolute substrate specificity (Givan, 1980). In leaf peroxisomes of *Spinacia oleracea* both GGAT and SGAT enzymes showed AGAT activity (Rehfeld and Tolbert, 1972), whereas SGAT from *Phaseolus vulgaris* showed no AGAT activity (Smith, 1973).

AGAT specific activity was high in comparison to those of other aminotransferases including GGAT (Fig. 3). Alanine may be an important amino donor for photorespiratory glycine synthesis. This view is supported by the work of Betsche (1983) who showed that labelled nitrogen from  $^{15}\text{N}$ -alanine was incorporated into glycine at a rate three times faster than from  $^{15}\text{N}$ -glutamate in *Avena sativa* leaf tissue, although uptake rates and total pool sizes of these substrates were similar.

The responses of aminotransferase activities from nitrogen-fed and nitrogen-starved *L. minor* have thus far been compared. In comparing nitrate and ammonium treatments GGAT, GPAT and AGAT specific activities were slightly greater in ammonium-fed plants (Fig. 3). The greatest difference occurred for GPAT, this being consistent with an experiment with plants transferred from nitrate to ammonium (data not shown) and is in accord with greater rates of  $^{14}\text{CO}_2$  incorporation into alanine, the amino product of GPAT activity, and larger pool sizes of this amino acid reported for ammonium-fed plants compared to nitrate treatments (Erismann and Kirk, 1969). Also, protein contents were greater in plants grown on ammonium than on nitrate (Fig. 1) which was previously shown in *L. minor* (Dicht, Kopp, Feller, and Erismann, 1976). This may arise from the absence of competition from nitrate reduction with anabolic processes for reducing power when ammonium is provided and/or from an additional supply of amino groups by alanine to an enhanced carbon flux through the PCO cycle (Emes and Erismann, 1982; Fuhrer and Erismann, 1984), which provides more amino acids for protein synthesis.

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## LITERATURE CITED

- BETSCH, T., 1983. Aminotransfer from alanine and glutamate to glycine and serine during photorespiration in oat leaves. *Plant Physiology*, **71**, 961-5.
- BLACKWOOD, G. C., and MIFLIN, B. J., 1976. The effect of nitrate and ammonium feeding on carbon dioxide assimilation in maize. *Journal of Experimental Botany*, **27**, 735-47.
- BRADFORD, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248-54.
- DAVIES, D. D., and HUMPHREY, T. J., 1978. Amino acid recycling in relation to protein turnover. *Plant Physiology*, **61**, 54-8.
- DICHT, M., KOPP, A., FELLER, U., and ERISMANN, K. H., 1976. Einfluss von Ammonium und Nitrat auf den Proteingehalt von *Lemna minor* L. unter Photosynthesebedingungen. *Biochemie und Physiologie der Pflanzen*, **170**, 531-4.
- EMES, M. J., and ERISMANN, K. H., 1982. The influence of the nitrogen supply on the structure and activity of glycolate oxidase in *Lemna minor* L. *Plant Science Letters*, **27**, 103-9.
- ERISMANN, K. H., and KIRK, M. R., 1969. The influence of nitrogen source on metabolic intermediates in steady-state photosynthesis by *Lemna minor* L. *Progress in Photosynthesis Research*, **3**, 1538-45.
- FUHRER, J., and ERISMANN, K. H., 1984. Steady-state carbon flow in photosynthesis and photorespiration in *Lemna minor* L.: the effect of temperature and ammonium nitrogen. *Photosynthetica*, **18**, 74-83.
- GIVAN, C. V., 1980. Aminotransferases in higher plants. In *The biochemistry of plants. A comprehensive treatise, volume 5, Amino acids and derivatives*. Eds P. K. Stumpf and E. E. Conn. Academic Press, New York, London. Pp. 329-57.
- HABIB MOHAMED, A., and GNANAM, A., 1977. Regulation of photosynthetic carbon flow by ammonium ions in isolated bean leaf cells. *The Plant Biochemical Journal*, **4**, 1-9.
- HEDLEY, C. L., and STODDART, J. L., 1971. Light-stimulation of alanine aminotransferase in dark-grown leaves of *Lolium temulentum* L. as related to chlorophyll formation. *Planta*, **100**, 309-24.
- HUTNER, S. H., 1953. Comparative physiology of heterotrophic growth in plants. In *Growth and differentiation in plants*. Ed. N. E. Loomis. Iowa State College Press, Ames, Iowa. Pp. 417-46.
- KEYS, A. J., BIRD, I. F., CORNELIUS, M. J., LEA, P. J., WALLSGROVE, R. M., and MIFLIN, B. J., 1978. Photorespiratory nitrogen cycle. *Nature*, **275**, 741-3.
- MARQUES, I. A., OBERHOLZER, M. J., and ERISMANN, K. H., 1983. Effects of different inorganic nitrogen sources on photosynthetic carbon metabolism in primary leaves of non-nodulated *Phaseolus vulgaris* L. *Plant Physiology*, **71**, 555-61.

- PLATT, S. G., PLAUT, Z., and BASSHAM, J. A., 1977. Ammonia regulation of carbon metabolism in photosynthesizing leaf discs. *Ibid.* **60**, 739-42.
- REHFELD, D. W., and TOLBERT, N. E., 1972. Aminotransferases in peroxisomes from spinach leaves. *Journal of Biological Chemistry*, **247**, 4803-11.
- RHODES, D., RENDON, G. A., and STEWART, G. R., 1976. Regulation of ammonia assimilating enzymes in *Lemna minor*. *Planta*, **129**, 203-10.
- SMITH, I. K., 1973. Purification and characterization of serine:glyoxylate aminotransferase from kidney bean (*Phaseolus vulgaris*). *Biochimica et biophysica acta*, **321**, 156-64.
- TISCHNER, R., and LORENZEN, R., 1980. Changes in the enzyme pattern in synchronous *Chlorella sorokiniana* caused by different nitrogen sources. *Zeitschrift für Pflanzenphysiologie*, **100**, 333-41.